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Synthesis of *p*-amino-WNA derivatives to enhance the stability of the anti-parallel triplex

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ABSTRACT

We have previously developed W-shaped nucleoside analogs (WNAs) having a nucleobase and an aromatic ring for the formation of the unnatural triplex DNA. Modification of an aromatic ring of WNA is highly effective regarding the stability of the triplex DNA. In this study, we designed new WNA analogs having the *p*-aminobenzene as an aromatic ring, which were synthesized via the Curtius–Yamada rearrangement. Based on the evaluation of the triplex formation with *p*-amino-WNA-TFO, it has been shown that the amino group may produce a non-selective interaction with the phosphate backbone of the target duplexes. These results indicate that the amino modification is useful to overcome the sequence-dependence of the TFO containing WNA analogs.

1. Introduction

Triplex DNA is useful for gene research, therapies, and biological tools by the gene inhibition[1] or activation[2] of the gene expression, gene recombination,[3] etc. In an anti-parallel triplex DNA, the purine-rich triplex forming oligonucleotides (TFOs) can bind to only the homopurine strand by two reverse Hoogsteen hydrogen bonds within the major groove of the duplex DNA in a sequence specific manner (G/GC and A/AT).[4] However, pyrimidine nucleosides, which insert into the purine strand, such as TA and CG base pairs, inhibit the stable triplex formation using the natural TFOs. These base pairs are called interrupting sites.

Recently, we developed nucleoside analogs (WNA: W-shaped nucleoside analogs) having a nucleobase as a recognition part and an aromatic ring as a stacking part.[5,6] It was demonstrated that the WNA-βT and WNA-βC exhibited selective recognition of a TA or a CG interrupting site, respectively (Fig. 1). Our previous studies have also shown that the recognition of the interrupting sites is dependent on the neighboring nucleobase of the WNA analogs in the TFOs.[9] In this study, we focused on the modification of the aromatic ring to solve this sequence dependency and synthesized a variety of WNA analogs having a modified aromatic ring. It was shown that bromo-substituted WNA-βT analogs, *o*Br-WNA-βT and *m*Br-WNA-βT, could stabilize a TA interrupting site with selectivity in sequences, which could not be stabilized by the original WNA-βT.[7–9] On the other hand, the *para*-bromo-substituted WNA analogs had no effect on the triplex stability. These results suggested that the triplex DNA was inhibited by the steric hindrance between the *p*-bromo group of WNA and the phosphate group of the target duplex DNA. Therefore, a functional group, such as an amino group, at the *para* position of the benzene ring may produce an attractive interaction with the phosphate group to enhance the stability of the triplex.[10] Thus, a new *p*-amino-substituted analog of WNA (*p*-amino-WNA) was designed. It is suggested by molecular modeling

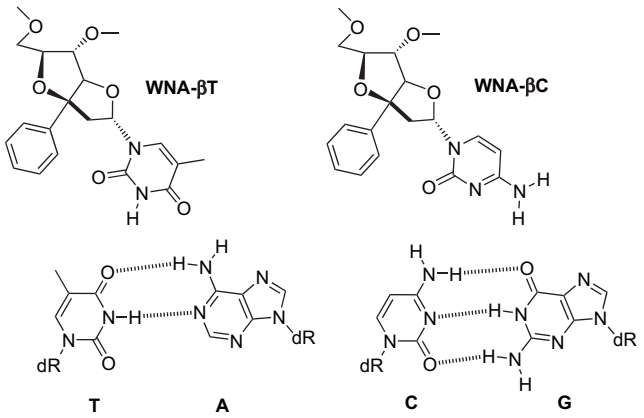

Figure 1. Speculated recognition model of WNA-βT/TA and WNA-βC combinations (WNA W-shaped nucleoside analogs).

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of the triplex with the base triplet of *p*-amino-WNA-βT and a TA base pair that the amino group is in close proximity to the phosphate group (Fig. 2). We now describe the synthesis and evaluation of the triplex forming ability of the new WNA analogs, *p*-amino-WNA-α, βT and -α, βC.

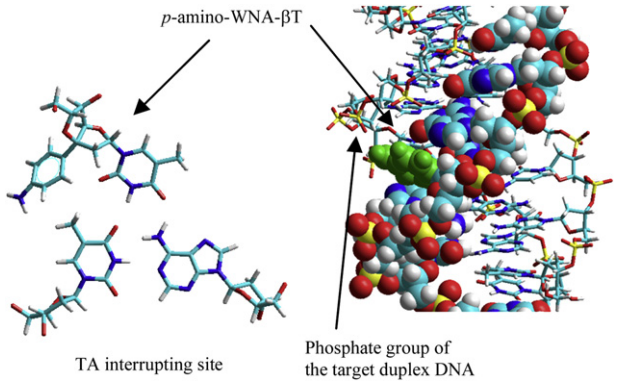
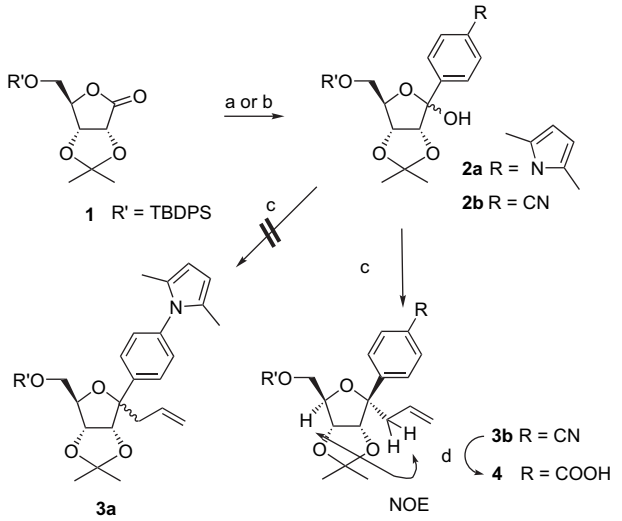


Figure 2. The predicted structure of triplex formation with TFO having *p*-amino-WNA-βT by MD calculation. The complex between *p*-amino-WNA-βT and TA interrupting site (left). TFO is shown with Space-Filling Model and *p*-aminobenzene shows green color (right).

2. Results and discussion

2.1. Synthesis of TFOs containing *p*-amino-WNA

According to our early method, in which the WNA analogs were synthesized through the Lewis acid-catalyzed allylation of the 1′-phenyl substituted ribose intermediate (**2**), the 2,5-dimethylpyrole substituted derivative (**2a**) was chosen as an intermediate (Scheme 1). D-Ribonolacton **1**[6] was treated with 1-(4-bromophenyl)-2,5-dimethylpyrrole[11] and *n*-BuLi at −78 °C to afford **2a** in 86% yield. However, in spite of attempts such as the reactions at higher temperature or in the presence of other Lewis acids, the allylation of **2a** was not successful. Alternatively, it was expected that the already-reported *p*-cyano derivative (**3b**)[8] might be transformed into the amino derivative via the Curtius rearrangement following hydrolysis. D-Ribonolacton **1** was treated with *p*-bromocyanobenzene and *n*-BuLi at −100 °C to afford **2b** in 79% yield. After the allylation reaction, the anomeric isomers were separated by column chromatography to obtain the α-allyl **3b** in 40% and the β-allyl compound in 56% yield. These isomers were determined by the ^{1}H COSY and NOESY spectra. The hydrolysis of the desired isomer **3b** gave the carboxylic acid **4** in 95% yield in three steps.[12,13] Next, we investigated the Yamada procedure using DPPA[14] for the Curtius rearrangement of **4** (Table 1). As an amino protecting group needs to be tolerable to acidic conditions included in the later synthesis (Scheme 2), the reaction was performed in the absence of alcohol to obtain the amino compound. The rearranged product **5a** was obtained, but in low yield together with the dimerized urea compound (Table 1, entry 1). In the presence of ethanol, the ethoxycarbonylamino compound (**5b**) was obtained in good yield (Table 1, entry 2), but this protecting group was not removed under basic or acidic conditions. The rearrangement was also successful in the presence of *tert*-butanol (Table 1, entry 4) and not in the presence of trifluoroethanol (Table 1, entry 3). Thus, an acid-labile *t*-Boc protecting group was converted to the acid-stable trifluoroacetyl group (Scheme 2). The Boc group was removed by TFA in CH_2Cl_2 at 0 °C to produce the corresponding amino compound, which was again protected with the trifluoroacetyl group to give the intermediate **6** in 91% yield in two steps. The trifluoroacetyl group is compatible to reagents and conditions used in an automated DNA synthesizer.[15] Subsequently, oxidative cleavage of the vinyl group of **6**, the cyclization reaction under the acidic condition, followed by protection of the hydroxyl group afforded the bicyclic intermediate **7** in a 64% overall yield. Nucleobases, thymine or *N*-benzoylcytosine, were coupled with this intermediate **7** using Lewis acids to give **8b**-**1** (βT) in 50%, **8a**-**1** (αT) in 47%, **9b**-**1** (βC) in 47%, and **9a**-**1** (αC) in 50% yield. These *N*-glycosylated compounds were separated by silica-gel column chromatography and their stereochemistries were determined by the ^{1}H COSY and NOESY spectra. Deprotection of TBDPS and the acetyl groups of the 5′- and 3′-hydroxyl groups gave the corresponding diol compounds (**8a**,**b**-**2**, **9a**,**b**-**2**) in 69–97% yield. These diol compounds were converted to the phosphoramidite precursor (βT: **8b**-**3** in 49%; αT: **8a**-**3** in 39%; βC: **9b**-**3** in 59%; and αC: **9a**-**3** in 35% yield). The TFOs (**TFO1**–**4**) containing *p*-amino-WNA derivatives were obtained in good yield using an automated DNA synthesizer. The **TFO1** in the sequence of 3′-AZG-5′ containing *p*-amino-WNA-αT at the position of Z was called **TFO1**(**αT**). After purification by HPLC, the purity and structure of these TFOs were identified by MALDI-TOF MS measurements.

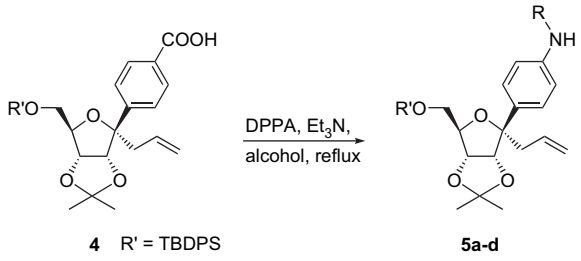


Scheme 1. Reagents and conditions: (a) 1-(4-bromophenyl)-2,5-dimethylpyrrole, *n*-BuLi, THF, −78 °C (86%). (b) 4-Bromobenzonitrile, *n*-BuLi, THF, −100 °C (79%). (c) Allyltrimethylsilane, $ZnBr_2$, CH_3NO_2, rt, (α-allyl: 40%, β-allyl: 56%). (d) (1) KOH, H_2O_2, EtOH, H_2O, reflux; (2) TBDPSCl, Et_3N, DMAP, CH_2Cl_2, rt; (3) K_2CO_3, H_2O, MeOH, THF, rt (95% for three steps).

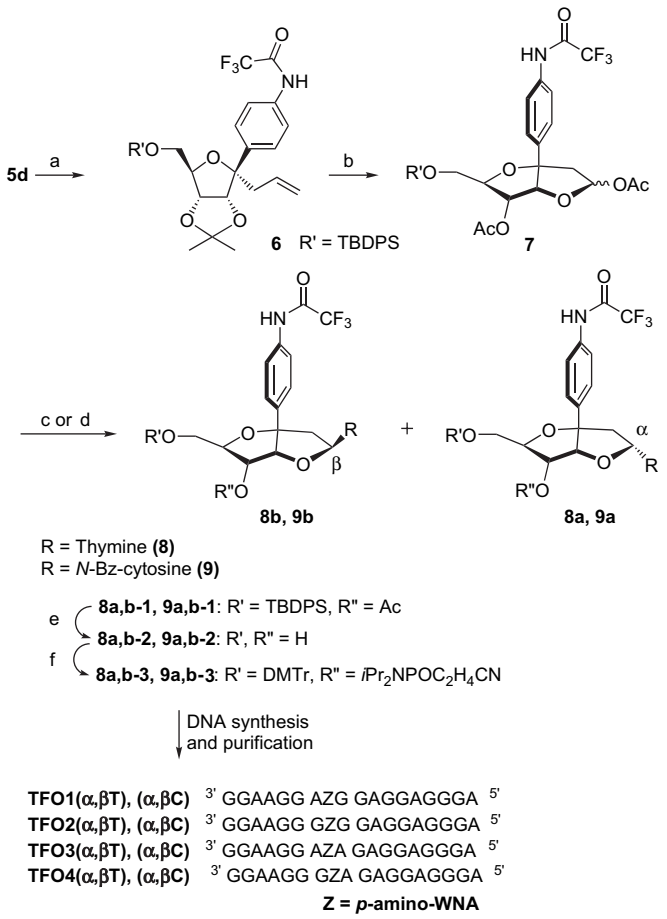
2.2. Triplex ability of TFOs containing WNA analogs

The ability of the triplex formations was evaluated by gel-shift assay using the ^{32}*P*-labeled TFO as a tracer. The association

Table 1
Conditions of Curtius–Yamada rearrangement



Entry	–R	Yield (%)
1	–H	50
2	$-COOCH_2CH_3$	87
3	$-COOCH_2CF_3$	10
4	$-COOC(CH_3)_3$	85



Scheme 2. Reagents and conditions: (a) (1) TFA, CH_2Cl_2, 0 °C; (2) $(CF_3CO)_2O$, pyridine, 0 °C; (3) acetone, TsOH, rt (91% for three steps). (b) (1) OsO_4 (aq), $NaIO_4$ (aq), pyridine, rt; (2) H_2SO_4 (aq), THF, 60 °C; (3) Ac_2O, pyridine, rt (64% for three steps). (c) Thymine, TMSOTf, CH_3CN, rt (97%). (d) Benzoylcytosine, BSA, TMSOTf, CH_3CN, rt (97%). (e) (1) TBAF, THF, rt; (2) 0.2 M NaOH (aq), MeOH, THF, 0 °C (69–97% for two steps). (f) (1) DMTrCl, pyridine; (2) $^{i}Pr_2NP(Cl)OCH_2CH_2CN$, DIPEA, CH_2Cl_2, 0 °C (35–59% for two steps). The naming of the TFOs: for example, TFO1(αT) represents the TFO1 sequence incorporating *p*-amino-WNA-αT at the Z position.

constants (K_s values) of the triplex formations were calculated from the intensity of the radioactive bands, as previously described.[6,9]

The results of the K_s values of **TFO1**-**4**(**β,αT**) containing Z=*p*-amino-WNA-βT and *p*-amino-WNA-αT are summarized in Figure 3A and B, respectively. **TFO1**(**βT**) having the *p*-amino-WNA-βT in the sequence of 3′-AZG-5′ had no stabilizing effect for the four target duplex DNAs (Fig. 3A, purple bar). **TFO2**(**βT**) consisting of G at the two neighboring sides of the *p*-amino-WNA-βT showed a stabilizing effect for the CG and GC base pairs (Fig. 3A, green bar). **TFO3**(**βT**) (3′-AZA′) and **TFO4**(**βT**) (3′-GZA-5′) also exhibited a non-selective stabilizing effect (Fig. 3A, yellow and pink bars). In Figure 3B, interestingly, **TFO1**–**4**(**αT**) having the *p*-amino-WNA-αT showed a similar selectivity with **TFO1**–**4**(**βT**) in each combination, although the magnitude of the K_s values was different.

The K_s values of *p*-amino-WNA-C are summarized in Figure 3C and D. **TFO1**(**βC**), **TFO2**(**βC**), and **TFO4**(**βC**) having a sequence of 3′-AZG-5′, 3′-GZG-5′, and 3′-GZA-5′ showed a non-selective stabilizing effect (Fig. 3C, purple, green, and pink bars). On the other hand, **TFO3**(**βC**) in the sequence of 3′-AZA-5′ showed selective triplex formations for the AT and GC base pairs (Fig. 3C, yellow bar). Similarly, **TFO1**(**αC**), **TFO2**(**αC**), and **TFO4**(**αC**) containing *p*-amino-WNA-αC showed the non-selective triplex formations, and **TFO3**(**αC**) (3′-AZA-5′) stabilized the AT and GC base pairs (Fig. 3D). Therefore, the selectivity of the β-isomer is similar to that of the α-isomer of *p*-amino-WNA-C (Fig. 3C and D).

The parent **WNA-βT** exhibited selective stabilization to the TA interrupting site in the sequence of **TFO1** and **2** (3′-AZG-5′ and 3′-GZG-5′, Z=**WNA-βT**).[9] Therefore, it was expected that the **TFO** containing the *p*-amino-substituted **WNA-βT** would also recognize the TA interrupting site. However, the *p*-amino-substituted **WNA-βT** series showed quite different recognition profiles, in which there was no stabilization by **TFO1** (3′-AZG-5′), CG stabilization by **TFO2** (3′-GZG-5), and stabilization to the AT and GC sites by **TFO3** (3′-AZA-5′) and **4** (3′-GZA-5) (Fig. 3A and B). It was speculated in our previous study that the thymine of **WNA-βT** might interact with the junction of the T–A base pair.[6] As the site for the formation of the base triplet is in a crowded environment as shown in Figure 2, the amino-phosphate interaction might distort this complex structure and induce a drastic change in the recognition profiles. A preferable complex structure formed between a TA site and the parent **WNA-βT** in **TFO1** might suffer from a subtle change, resulting in loss of overall affinity. The parent **WNA-βC** stabilized a CG site only in the sequence **TFO1**(3′-AZG-5′),[9] in contrast, the *p*-amino derivatives of **WNA-C** showed non-specific stabilization (Fig. 3C and D). It was reported that the sequence dependency of non-natural nucleobases in a parallel triplex was attributable to stacking interactions between the non-natural nucleobase and the bases of the TFO and the target DNA strands.[16] Similarly, stacking interactions may contribute to the sequence dependency of the WNA analogs, because **WNA-H**, lacking a nucleobase, showed sequence dependent triplex stabilization.[6,9] Surprisingly, although the recognition base of the WNA analogs and the magnitude of the K_s value are different, both stereochemistries α and β produced a similar selectivity to the target base pair (Fig. 3A vs B and Fig. 3C vs D). Triplex stabilization is made by harmonization of various factors such as hydrogen bonds, stacking interactions, shape complementarity, and so on. The results of this study have clearly indicated that the interaction of the amino group of the WNA analogs with the phosphate backbone of the target DNA can be an additional stabilizing effect of the triplex.

3. Conclusion

In conclusion, we designed and synthesized *p*-amino modified WNA analogs. The evaluation of the triplex forming ability showed that the *p*-amino-WNA analogs provide a non-selective stabilizing effect for the triplex DNA despite the different recognition bases such as a thymine and a cytosine and a different stereochemistry. These results indicated that the amino group might interact with the phosphate group of the target duplex DNA in close proximity. Therefore, the modification of the *para* position at the aromatic part of the WNA analogs has a potential enhancement effect on the stability of the triplex DNA. Further studies involving other modifications of the *para* position are now ongoing.

4. Experimental section

4.1. General

The ^1H NMR (400, 500 MHz) and ^{13}C NMR (100, 125 MHz) spectra were recorded by Varian UNITY-400 and INOVA-500 spectrometers, respectively. ^{31}P NMR (161 MHz) spectrum was recorded using 10% phosphoric acid in D_2O for the internal standard at 0 ppm. IR spectra were obtained using a PerkinElmer FTIR-SpectrumOne. High-resolution mass spectra were recorded on an Applied Biosystems Mariner System 5299 spectrometer.

*4.1.1. (1R,5R,6S,8R)-3,3-Dimethyl-6-(p-cyanophenyl)-6-(2′-propenyl)-8-(tert-butyldiphenylsilyloxymethyl)-2,4,7-trioxabicyclo[3.3.0]octane (**3b**)*

To a solution of 4-bromobenzonitrile (0.85 g, 4.69 mmol) in THF (18 mL) was added *n*-BuLi (1.6 M in hexane solution, 3.0 mL,

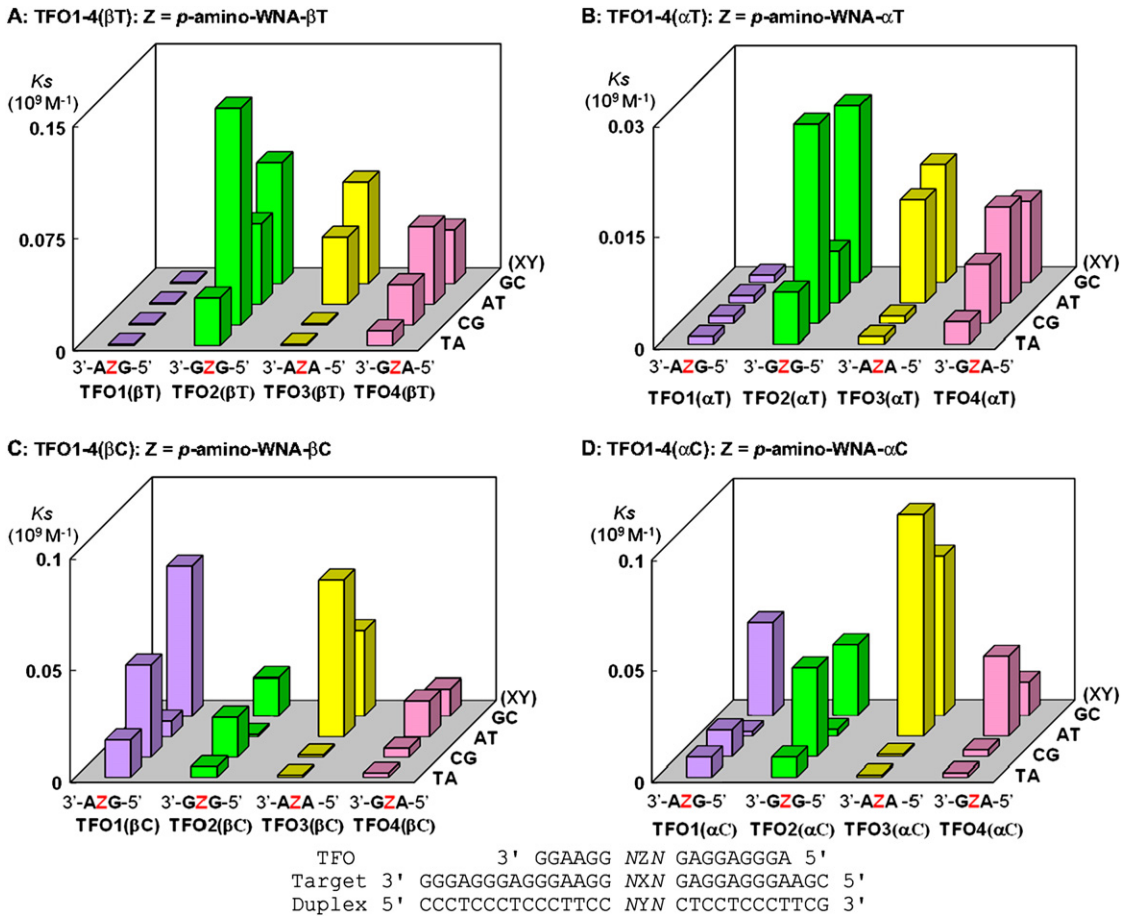


Figure 3. Bar graph of the association constant (K_s) by TFOs containing *p*-amino-WNA derivatives. Conditions: triplex formation was done for 12 h at 22 °C in the buffer containing 20 mM Tris–HCl, 5 mM $MgCl_2$, 2.5 mM spermidine, and 10% sucrose at pH 7.5. Electrophoresis was done at 10 °C with 15% non-denatured polyacrylamide gel. TFO (10 nM, 18mer) containing the ^{32}P-labeled one as the tracer was used. The concentration of the target duplex (30 bp) was increased (0–100 nM). The K_s values of the natural G/GC triplet using the natural TFO (Z=dG) are 0.086×10^9 (3′-AGG-5′), 0.66×10^9 (3′-GGG-5′), 0.58×10^9 (3′-AGA-5′), and 0.69×10^9 (3′-GGA-5′).[9]

4.69 mmol) at −100 °C. This mixture was stirred at the same temperature for 1 h. A solution of **1** (1.0 g, 2.34 mmol) in THF (18 mL) was added to this mixture. After stirring for 2.5 h, the reaction was quenched with a satd NH_4Cl solution and extracted with EtOAc. The organic solvent was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by silica-gel column chromatography (hexane/EtOAc=9:1) to give **2b** as a colorless foam (0.98 g, 1.86 mmol, 79%; HRMS (ESI) *m*/*z*: calcd for $C_{31}H_{35}NO_5SiNa$ $(M+Na)^+$ 552.2177, found 552.2173). A solution of **2b** (0.92 g, 1.73 mmol) in CH_3CN (4.6 mL) and allyltrimethylsilane (1.67 mL, 10.38 mmol) was added to a suspension of zinc bromide (1.33 g, 5.88 mmol) at 0 °C. After stirring for 3 h at room temperature, the reaction was quenched with a satd $NaHCO_3$ solution and extracted with EtOAc. The organic solvent was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by silica-gel column chromatography (hexane/EtOAc=17:1) to give **3b** as a colorless oil (α-allyl, 0.38 g, 0.69 mmol, 40%) and the other isomer as a colorless oil (β-allyl, 0.97 mmol, 56%). *Compound of α-allyl*. ^1H NMR (400 MHz, $CDCl_3$): δ 7.62–7.59 (4H, m), 7.52 (2H, d, *J*=8.2 Hz), 7.46 (2H, d, *J*=8.2 Hz), 7.43–7.39 (2H, m), 7.36–7.32 (4H, m), 5.53–5.43 (1H, m), 4.89–4.80 (2H, m), 4.71 (1H, dd, *J*=7.0, 3.4 Hz), 4.62 (1H, d, *J*=7.0 Hz), 4.25 (1H, q, *J*=4.3 Hz), 3.77 (2H, d, *J*=4.3 Hz), 2.77 (1H, dd, *J*=14.7, 7.0 Hz), 2.52 (1H, dd, *J*=14.7, 7.3 Hz), 1.61 (3H, s), 1.35 (3H, s), 0.98 (9H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 149.9, 135.6, 133.22, 132.5, 131.6, 129.8, 127.7, 126.4, 118.9, 118.2, 114.9, 110.6, 87.6, 87.5, 82.2, 82.1, 64.1, 40.5, 26.8, 26.0, 24.9, 19.2. FTIR (neat): 3074, 2931, 2858, 2229, 1739 cm^{-1}. HRMS (ESI) *m*/*z*: calcd for $C_{34}H_{39}NO_4SiNa$ $(M+Na)^+$ 576.2541, found 576.2519.

*4.1.2. (1R,5R,6S,8R)-3,3-Dimethyl-6-(p-carboxyphenyl)-6-(2′-propenyl)-8-(tert-butyldiphenylsilyloxymethyl)-2,4,7-trioxabicyclo[3.3.0]octane (**4**)*

A solution of **3b** (3.92 g, 7.09 mmol) in CH_3CH_2OH (30 mL), 40% KOH solution (35 mL) and 30% H_2O_2 (5 mL) were refluxed for 21 h. The reaction mixture was cooled at 0 °C and acidified using a 23% HCl solution. This solution was extracted with EtOAc. The organic solvent was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue dissolved in CH_2Cl_2 (25 mL), Et_3N (2.37 mL, 17 mmol), *tert*-butylchlorodiphenylsilane (4.36 mL, 17 mmol), and DMAP (0.21 g, 1.7 mmol) were stirred for 3 h. This reaction mixture was diluted with EtOAc, and washed with a satd NH_4Cl solution, water, and brine. The organic layer was dried over Na_2SO_4 and evaporated. The residue dissolved in THF (30 mL), CH_3OH (10 mL), and a K_2CO_3 solution (2.94 g in water (15 mL), 21.3 mmol) were stirred for 13 h. The reaction was quenched with a 10% HCl solution and extracted with EtOAc. The organic solvent was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by silica-gel chromatography (hexane/EtOAc=5:1 to 2:1) to give **4** as a colorless foam (3.84 g, 6.70 mmol, 95%). ^1H NMR (400 MHz, $CDCl_3$): δ 8.00 (2H, d, *J*=8.6 Hz), 7.65–7.60 (4H, m), 7.48 (2H, d, *J*=8.6 Hz), 7.43–7.32 (6H, m), 5.57–5.47 (1H, m), 4.89–4.83 (2H, m), 4.72–4.68 (2H, m), 4.26 (1H, q, *J*=4.6 Hz), 3.79 (1H, dd, *J*=11.0, 4.6 Hz), 3.75 (1H, dd, *J*=11.0, 4.6 Hz), 2.79 (1H, dd, *J*=14.7,

7.0 Hz), 2.57 (1H, dd, *J*=14.7, 7.3 Hz), 1.62 (3H, s), 1.36 (3H, s), 0.99 (9H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.4, 150.7, 135.6, 133.2, 132.8, 129.8, 129.7, 127.7, 125.7, 118.0, 114.7, 87.7, 82.2, 64.2, 40.7, 26.8, 26.0, 25.0, 19.2. FTIR (neat): 2931, 1689 cm^{-1}. HRMS (ESI) *m*/*z*: calcd for $C_{34}H_{40}O_6SiNa$ $(M+Na)^+$ 595.2486, found 595.2533.

4.1.3. *(1R,5R,6S,8R)-3,3-Dimethyl-6-(p-trifluoroacetyl-aminophenyl)-6-(2′-propenyl)-8-(tert-butyldiphenylsilyl-oxymethyl)-2,4,7-trioxabicyclo[3.3.0]octane (**6**)*

The solution of **4** (6.0 g, 10.5 mmol) in *tert*-BuOH (220 mL), EtN_3 (2.9 mL, 21 mmol), and DPPA (diphenylphosphoryl azide, 4.5 mL, 21 mmol) was refluxed for 20 h. The reaction mixture was diluted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by silica-gel chromatography (hexane/Et_2O=10:1) to give **5d** as a colorless oil (5.8 g, 8.93 mmol, 85%). This residue (5.7 g, 8.88 mmol) was dissolved in CH_2Cl_2 (76 mL) and trifluoroacetic acid (19 mL) at 0 °C. After stirring for 2.5 h, this reaction mixture was quenched with satd $NaHCO_3$ solution and extracted with EtOAc. This organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated. The solution of this residue in pyridine (145 mL) and trifluoroacetic anhydride (5.0 mL, 35.6 mmol) was stirred for 1.5 h at 0 °C. The reaction mixture was diluted with satd $NaHCO_3$ and extracted with EtOAc. This organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated. This residue was dissolved in acetone (40 mL) and *p*-toluenesulfonic acid monohydrate (50.8 mg, 0.26 mmol). After stirring for 90 h, this reaction mixture was quenched with $NaHCO_3$ (80 mg, 0.98 mmol), the solids were filtered off, and then the filtrate was evaporated. The residue was purified by silica-gel chromatography (hexane/Et_2O=6:1) to give **6** as colorless oil (5.16 g, 8.06 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (1H, s), 7.64–7.61 (4H, m), 7.45–7.32 (10H, m), 5.58–5.47 (1H, m), 4.89–4.83 (2H, m), 4.71–4.65 (2H, m), 4.23 (1H, q, *J*=4.6 Hz), 3.77 (1H, dd, *J*=11.0, 4.9 Hz), 3.73 (1H, dd, *J*=11.0, 4.6 Hz), 2.76 (1H, dd, *J*=14.7, 7.0 Hz), 2.54 (1H, dd, *J*=14.6, 7.3 Hz), 1.61 (3H, s), 1.35 (3H, s), 1.00 (9H, s). FTIR (neat): 3302, 2933, 2859, 1709 cm^{-1}. HRMS (ESI) *m*/*z*: calcd for $C_{35}H_{40}F_3NO_5SiNa$ $(M+Na)^+$ 662.2520, found 662.2531.

4.1.4. *(1R,3R,4R,5R,7RS)-1-(p-Trifluoroacetylaminophenyl)-3-(tert-butyldiphenylsilyloxymethyl)-4,7-diacetoxy-2,6-dioxabicyclo-[3.3.0]octane (**7**)*

This detailed procedure was described in Ref. 6. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83 (0.5H, s), 7.77 (0.5H, s), 7.69–7.60 (5H, m), 7.55 (1H, d, *J*=8.55 Hz), 7.49 (1H, d, *J*=8.55 Hz), 7.47 (1H, d, *J*=8.9 Hz), 7.45–7.31 (6H, m), 6.62 (0.5H, d, *J*=4.3 Hz), 6.50 (0.5H, d, *J*=5.8 Hz), 5.03 (0.5H, dd, *J*=9.2, 5.2 Hz), 5.02 (0.5H, dd, *J*=9.5, 4.3 Hz), 4.89 (0.5H, d, *J*=5.2 Hz), 4.77 (0.5H, d, *J*=4.3 Hz), 4.51 (0.5H, dt, *J*=9.2, 3.1 Hz), 4.20 (0.5H, dt, *J*=9.5, 2.4 Hz), 4.06 (0.5H, dd, *J*=11.9, 2.4 Hz), 4.05 (0.5H, dd, *J*=11.9, 2.7 Hz), 3.76 (0.5H, dd, *J*=11.6, 3.2 Hz), 3.73 (0.5H, dd, *J*=11.6, 3.2 Hz), 2.83 (0.5H, dd, *J*=15.0, 5.8 Hz), 2.71 (0.5H, dd, *J*=15.0, 5.8 Hz), 2.60 (0.5H, dd, *J*=15.3, 1.5 Hz), 2.57 (0.5H, d, *J*=15.0 Hz), 2.13 (1.5H, s), 2.07 (1.5H, s), 2.02 (3H, s), 1.02 (9H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.0, 169.7, 139.9, 135.6, 134.4, 133.1, 132.9, 129.8, 127.8, 127.7, 126.4, 126.3, 120.4, 120.3, 99.5, 91.4, 88.8, 87.2, 80.1, 78.7, 72.3, 71.1, 62.7, 61.9, 50.4, 49.0, 29.7, 26.8, 21.3, 20.6, 20.5, 19.2. FTIR (neat): 3302, 2932, 1731 cm^{-1}. HRMS (ESI) *m*/*z*: calcd for $C_{35}H_{48}F_3NO_8SiNa$ $(M+Na)^+$ 708.2211, found 708.2222.

4.1.5. *(1′S,3′R,4′R,5′R,7′S and R)-{4′-Acetoxy-1′-(p-trifluoro-acetylaminophenyl)-3′-(tert-butyldiphenylsilyloxymethyl)-2′,6′-dioxabicyclo[3.3.0]oct-7′-yl}-thymines (**8b-1** and **8a-1**)*

N,*O*-Bis(trimethylsilyl)acetamide (0.54 mL, 2.19 mmol) was added to a suspension of thymine (138 mg, 1.1 mmol) in CH_3CN (12 mL). A solution of TMSOTf (0.2 mL, 1.1 mmol) and **7** (500 mg, 0.73 mmol) in CH_3CN (7.0 mL) was added to the above mixture. After stirring for 3 h, the reaction mixture was quenched with a satd $NaHCO_3$ solution and extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated. The isomers were separated by flash silica-gel column chromatography (hexane/$CHCl_3$/acetone=5:5:2 to 2:2:1) to give each isomer in a total of 97% yield. *p*-Amino-WNA-βT (**8b-1**): a white powder (276 mg, 0.37 mmol, 50%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.62 (1H, s), 8.46 (1H, s), 7.71 (2H, d, *J*=8.5 Hz), 7.63 (2H, dd, *J*=7.9, 1.2 Hz), 7.58 (2H, dd, *J*=7.9, 1.2 Hz), 7.51 (2H, d, *J*=8.5 Hz), 7.44–7.30 (6H, m), 7.11 (1H, d, *J*=1.2 Hz), 6.01 (1H, t, *J*=6.7 Hz), 5.27 (1H, d, *J*=3.7 Hz), 5.10 (1H, dd, *J*=9.2, 3.7 Hz), 4.27 (1H, dt, *J*=9.2, 3.4 Hz), 4.00 (1H, dd, *J*=12.0, 3.1 Hz), 3.72 (1H, dd, *J*=12.0, 3.7 Hz), 2.85 (1H, dd, *J*=14.0, 6.7 Hz), 2.77 (1H, dd, *J*=14.0, 6.7 Hz), 2.05 (3H, s), 1.94 (3H, s), 0.99 (9H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9, 164.2, 150.7, 137.2, 135.6, 135.5, 135.4, 133.0, 132.9, 129.8, 127.8, 127.7, 126.4, 120.3, 111.1, 92.9, 87.4, 80.2, 73.4, 62.7, 48.1, 29.7, 26.7, 20.7, 19.2, 12.4. FTIR (neat): 3262, 3070, 2929, 2856, 2336, 1688 cm^{-1}. HRMS (ESI) *m*/*z*: calcd for $C_{38}H_{41}F_3N_3O_8Si$ $(M+H)^+$ 752.2610, found 752.2595. *p*-Amino-WNA-αT (**8a-1**): a white powder (260 mg, 0.35 mmol, 47%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (1H, s), 7.91 (1H, s), 7.63–7.60 (4H, m), 7.55 (1H, d, *J*=1.22 Hz), 7.49 (4H, s), 7.45–7.33 (6H, m), 6.52 (1H, dd, *J*=7.9, 5.8 Hz), 4.99 (1H, dd, *J*=8.9, 4.0 Hz), 4.77 (1H, d, *J*=4.0 Hz), 4.33 (1H, quintet, *J*=4.0 Hz), 3.94 (1H, dd, *J*=11.6, 3.4 Hz), 3.76 (1H, dd, *J*=11.6, 4.3 Hz), 2.83 (1H, dd, *J*=15.3, 7.9 Hz), 2.61 (1H, dd, *J*=15.3, 5.8 Hz), 2.03 (3H, s), 1.95 (3H, s), 1.01 (9H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.9, 163.3, 150.5, 138.4, 135.6, 135.5, 134.9, 133.0, 132.8, 129.9, 129.8, 127.8, 126.2, 120.7, 112.0, 92.2, 85.9, 85.5, 80.3, 72.7, 63.0, 48.0, 29.7, 26.8, 20.6, 19.2, 12.8. FTIR (neat): 2958, 2923, 2853, 2331, 1694 cm^{-1}. HRMS (ESI) *m*/*z*: calcd for $C_{38}H_{41}F_3N_3O_8Si$ $(M+H)^+$ 752.2610, found 752.2631.

4.1.6. *(1′S,3′R,4′R,5′R,7′S and R)-N^4-Benzoyl-1-{4′-acetoxy-1′-(p-trifluoroacetylaminophenyl)-3′-(tert-butyldiphenylsilyloxymethyl)-2′,6′-dioxabicyclo-[3.3.0]oct-7′-yl}-cytosines (**9b-1** and **9a-1**)*

N,*O*-Bis(trimethylsilyl)acetamide (0.54 mL, 2.19 mmol) was added to a suspension of *N*-benzoylcytosine (235 mg, 1.1 mmol) in CH_3CN (12 mL). A solution of TMSOTf (0.2 mL, 1.1 mmol) and **7** (500 mg, 0.73 mmol) in CH_3CN (7.0 mL) was added to the above mixture. After stirring for 3 h, the reaction mixture was quenched with satd $NaHCO_3$ solution and extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated. The isomers were separated by flash silica-gel column chromatography (hexane/$CHCl_3$/acetone=2:2:1 to 1:1:1) to give each isomer in a total of 97% yield. *p*-Amino-WNA-βC (**9b-1**): a white powder (287 mg, 0.34 mmol, 47%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.75 (1H, s), 7.98 (1H, s), 7.94–7.88 (3H, m), 7.66–7.59 (8H, m), 7.52–7.33 (10H, m), 6.28 (1H, t, *J*=7.0 Hz), 5.16 (1H, d, *J*=4.0 Hz), 5.05 (1H, dd, *J*=9.2, 4.0 Hz), 4.27 (1H, dt, *J*=9.2, 3.4 Hz), 4.03 (1H, dd, *J*=11.6, 2.8 Hz), 3.75 (1H, dd, *J*=11.6, 3.4 Hz), 3.17 (1H, dd, *J*=14.5, 7.0 Hz), 2.59 (1H, dd, *J*=14.5, 7.0 Hz), 2.04 (3H, s), 1.01 (9H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.3, 162.5, 154.9, 154.6, 144.5, 137.7, 135.6, 135.0, 133.3, 133.1, 132.9, 129.9, 129.8, 129.1, 127.8, 127.6, 126.5, 120.5, 92.4, 91.1, 87.4, 80.4, 73.0, 62.4, 49.4, 29.7, 29.3, 26.8. FTIR (neat): 2928, 2855, 1731, 1703, 1660 cm^{-1}. HRMS (ESI) *m*/*z*: calcd for $C_{44}H_{44}F_3N_4O_8Si$ $(M+H)^+$ 841.2875, found 841.2925. *p*-Amino-WNA-αC (**9a-1**): a white powder (304 mg, 0.36 mmol, 50%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.74 (1H, s), 8.22 (1H, d, *J*=7.63 Hz), 8.03 (1H, s), 7.90 (2H, d, *J*=7.33 Hz), 7.63–7.57 (6H, m), 7.53–7.39 (8H, m), 7.37–7.33 (4H, m), 6.36 (1H, dd, *J*=7.3, 5.0 Hz), 5.05 (1H, dd, *J*=8.2, 4.6 Hz), 4.94 (1H, d, *J*=4.6 Hz), 4.21 (1H, dt, *J*=8.2, 4.3 Hz), 3.82 (1H, dd, *J*=11.6, 4.3 Hz), 3.73 (1H, dd, *J*=11.6, 4.3 Hz), 3.05 (1H, dd, *J*=15.3, 7.3 Hz), 2.66 (1H, dd, *J*=15.3, 5.0 Hz), 2.08 (3H, s),1.00 (9H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.8, 162.4, 155.0, 154.7, 143.8, 138.3, 135.6, 135.5, 135.1, 133.3, 132.9, 132.8, 129.9, 129.8, 129.1,

127.8, 127.6, 126.1, 120.7, 92.4, 88.5, 87.0, 80.4, 73.6, 63.0, 53.9, 49.5, 29.7, 29.3, 26.8. FTIR (neat): 2924, 2860, 1703, 1656, 1618 cm^{-1}. HRMS (ESI) *m*/*z*: calcd for $C_{44}H_{44}F_3N_4O_8Si$ $(M+H)^+$ 841.2875, found 841.2907.

4.1.7. General procedure of deprotection reaction

A THF solution of the above compound and TBAF (1.0 M THF solution, 3 equiv) were stirred for 3.5 h at room temperature. MeOH and a 0.2 M NaOH solution (4 equiv) were then added to this mixture at 0 °C. After stirring for 2.5 h at 0 °C, the reaction was quenched with acetic acid and diluted with MeOH. The solvents were removed under reduced pressure. The residue was purified by flash silica-gel column chromatography ($CHCl_3/CH_3OH$=13:1 to 9:1) to give the corresponding diol compound.

*4.1.8. (1′S,3′R,4′R,5′R,7′S)-{1′-(p-Trifluoroacetylaminophenyl)-4′-hydroxy-3′-hydroxymethyl-2′,6′-dioxabicyclo[3.3.0]oct-7′-yl}-thymine (**8b**-**2**)*

A colorless foam (97%). ^{1}H NMR (400 MHz, CD_3OD): δ 7.74 (2H, d, *J*=8.3 Hz), 7.69 (1H, d, *J*=1.0 Hz), 7.64 (2H, d, *J*=8.3 Hz), 6.27 (1H, t, *J*=8.3 Hz), 4.84 (1H, d, *J*=3.7 Hz), 4.03–3.99 (1H, m), 3.91–3.88 (2H, m), 3.68 (1H, dd, *J*=12.1, 5.5 Hz), 2.75–2.73 (2H, m), 1.93 (3H, d, *J*=1.0 Hz). ^{13}C NMR (125 MHz, CD_3OD): δ 166.4, 152.3, 139.3, 139.0, 137.1, 127.4, 122.0, 111.8, 93.0, 90.5, 90.3, 84.7, 73.6, 63.3, 12.3. FTIR (neat): 3267, 3074, 2922, 1686 cm^{-1}. HRMS (ESI) *m*/*z*: calcd for $C_{20}H_{21}F_3N_3O_7$ $(M+H)^+$ 472.1326, found 472.1364.

*4.1.9. (1′S,3′R,4′R,5′R,7′R)-{1′-(p-Trifluoroacetylaminophenyl)-4′-hydroxy-3′-hydroxymethyl-2′,6′-dioxabicyclo[3.3.0]oct-7′-yl}-thymine (**8a**-**2**)*

A colorless foam (69%). ^{1}H NMR (400 MHz, CD_3OD): δ 7.92 (1H, d, *J*=1.0 Hz), 7.67–7.63 (4H, m), 6.58 (1H, dd, *J*=8.0, 5.3 Hz), 4.53 (1H, d, *J*=3.9 Hz), 4.15–4.11 (1H, m), 3.88 (1H, dd, *J*=8.7, 3.9 Hz), 3.85 (1H, dd, *J*=12.1, 2.5 Hz), 3.68 (1H, dd, *J*=12.1, 6.6 Hz), 2.90 (1H, dd, *J*=15.1, 8.0 Hz), 2.68 (1H, dd, *J*=15.1, 5.3 Hz), 1.94 (3H, d, *J*=1.0 Hz). ^{13}C NMR (125 MHz, CD_3OD): δ 166.3, 152.7, 140.3, 138.3, 137.1, 127.2, 122.2, 112.2, 93.1, 90.3, 87.1, 84.1, 73.3, 63.7, 12.6. FTIR (neat): 2924, 2860, 1703, 1656, 1618 cm^{-1}. HRMS (ESI) *m*/*z*: calcd for $C_{20}H_{21}F_3N_3O_7$ $(M+H)^+$ 472.1326, found 472.1361.

*4.1.10. (1′S,3′R,4′R,5′R,7′S)-N^4-Benzoyl-1-{1′-(p-trifluoroacetylaminophenyl)-4′-hydroxy-3′-hydroxymethyl-2′,6′-dioxabicyclo[3.3.0]oct-7′-yl}-cytosine (**9b**-**2**)*

A colorless foam (92%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.40 (1H, d, *J*=7.6 Hz), 7.99 (2H, d, *J*=7.6 Hz), 7.74 (2H, d, *J*=8.9 Hz), 7.69–7.62 (4H, m), 7.56–7.52 (2H, m), 6.35–6.32 (1H, m), 4.94 (1H, d, *J*=3.7 Hz), 4.07–4.04 (1H, m), 3.95–3.91 (2H, m), 3.70 (1H, dd, *J*=12.2, 5.5 Hz), 3.00 (1H, dd, *J*=14.0, 5.8 Hz), 2.72 (1H, dd, *J*=14.0, 7.9 Hz). ^{13}C NMR (125 MHz, CD_3OD): δ 169.2, 165.0, 157.7, 150.1, 147.2, 139.3, 137.1, 134.7, 134.1, 129.8, 129.2, 127.4, 122.1, 98.7, 93.1, 92.4, 91.4, 84.4, 73.5, 63.2, 50.2. FTIR (neat): 3275, 2931, 1705, 1651 cm^{-1}. HRMS (ESI) *m*/*z*: calcd for $C_{26}H_{24}F_3N_4O_7$ $(M+H)^+$ 561.1592, found 561.1582.

*4.1.11. (1′S,3′R,4′R,5′R,7′R)-N^4-Benzoyl-1-{1′-(p-trifluoroacetylaminophenyl)-4′-hydroxy-3′-hydroxymethyl-2′,6′-dioxabicyclo[3.3.0]oct-7′-yl}-cytosine (**9a**-**2**)*

A colorless foam (95%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.66 (1H, d, *J*=7.7 Hz), 7.99 (2H, d, *J*=7.7 Hz), 7.69–7.62 (6H, m), 7.56–7.52 (2H, m), 6.49 (1H, dd, *J*=7.6, 4.7 Hz), 4.71 (1H, d, *J*=4.3 Hz), 4.11–4.07 (1H, m), 3.97 (1H, dd, *J*=8.6, 4.3 Hz), 3.82 (1H, dd, *J*=11.9, 2.4 Hz), 3.65 (1H, dd, *J*=11.9, 6.4 Hz), 3.07 (1H, dd, *J*=15.0, 7.6 Hz), 2.71 (1H, dd, *J*=15.0, 4.7 Hz). ^{13}C NMR (125 MHz, CD_3OD): δ 169.0, 164.7, 146.2, 140.3, 134.8, 133.9, 129.7, 129.2, 127.1, 122.1, 98.3, 93.0, 91.4, 89.7, 84.5, 73.5, 63.6, 50.5. FTIR (neat): 3318, 2925, 1702, 1633 cm^{-1}. HRMS (ESI) *m*/*z*: calcd for $C_{26}H_{24}F_3N_4O_7$ $(M+H)^+$ 561.1592, found 561.1631.

4.1.12. General procedure for the synthesis of phosphoramidite

DMTrCl (2.3 equiv) was added to a solution of the diol compound in pyridine. After stirring for 1 h, the reaction mixture was diluted with EtOAc, and then washed with water and brine. The organic layer was washed with water and brine, dried over Na_2SO_4, evaporated, and then the residue was purified by flash silica-gel column chromatography ($CHCl_3/CH_3OH$=99:1 containing 0.5% pyridine) to give the corresponding DMTr-protected WNA. ^{i}Pr$_2$NEt (6 equiv) was added to the solution of the above DMTr–WNA and ^{i}Pr$_2$NP(Cl)OC$_2$H$_4$CN (3 equiv) in CH_2Cl_2 at 0 °C. After stirring for 1 h, the reaction mixture was diluted with a satd $NaHCO_3$ solution and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated, and then the residue was purified by flash silica-gel column chromatography (hexane/EtOAc=1:1) to give the purified materials.

*4.1.13. (1′S,3′R,4′R,5′R,7′S)-{1′-(p-Trifluoroacetylaminophenyl)-3′-dimethoxytrityloxymethyl-4′-O-(N,N-diisopropyl-β-cyanoethyl-phosphoramidyl)-2′,6′-dioxabicyclo[3.3.0]oct-7′-yl}-thymine (**8b**-**3**)*

A colorless foam (49%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92 (1H, s), 7.65 (2H, d, *J*=8.9 Hz), 7.46 (2H, dd, *J*=8.9, 3.1 Hz), 7.43–7.39 (2H, m), 7.31–7.19 (8H, m), 6.79 (4H, dd, *J*=9.2, 2.8 Hz), 6.32 (0.5H, dd, *J*=8.2, 5.8 Hz), 6.13–6.10 (0.5H, m), 5.02 (0.5H, d, *J*=3.7 Hz), 4.91 (0.5H, d, *J*=3.4 Hz), 4.37–4.31 (0.5H, m), 4.24–4.22 (1H, m), 4.17–4.11 (0.5H, m), 3.83–3.62 (1H, m), 3.78 (3H, s), 3.77 (3H, s), 3.55–3.37 (4H, m), 3.20 (1H, dd, *J*=10.7, 4.6 Hz), 2.96–2.90 (1H, m), 2.69–2.64 (0.5H, m), 2.60–2.54 (1H, m), 2.52–2.46 (0.5H, m), 2.37–2.32 (1H, m), 1.98 (1.5H, s), 1.94 (1.5H, s), 1.12 (3H, d, *J*=6.7 Hz), 1.07–1.04 (6H, m), 0.90 (3H, d, *J*=6.7 Hz). ^{31}P NMR (161 MHz, $CDCl_3$): δ 150.0, 149.3. ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.5, 163.4, 158.5, 150.0, 149.8, 144.6, 144.5, 138.3, 138.1, 137.0, 136.3, 136.0, 135.9, 134.7, 134.6, 130.3, 130.2, 128.4, 127.8, 126.8, 126.6, 120.6, 118.0, 117.5, 113.1, 111.4, 110.9, 92.0, 91.9, 90.6, 88.8, 88.0, 86.2, 81.3, 81.2, 74.2, 74.0, 72.7, 72.5, 63.0, 62.0, 58.8, 58.7, 58.0, 57.8, 55.3, 55.2, 48.3, 47.9, 43.4, 43.3, 43.2, 24.7, 24.6, 24.5, 24.4, 24.3, 20.4, 20.3, 20.1, 12.5. FTIR (neat): 3289, 2964, 2932, 1690 cm^{-1}. ESI-MS *m*/*z*: 974 $(M+H)^+$, 996 $(M+Na)^+$.

*4.1.14. (1′S,3′R,4′R,5′R,7′R)-{1′-(p-Trifluoroacetylaminophenyl)-3′-dimethoxytrityloxymethyl-4′-O-(N,N-diisopropyl-β-cyanoethyl-phosphoramidyl)-2′,6′-dioxabicyclo[3.3.0]oct-7′-yl}-thymine (**8a**-**3**)*

A colorless foam (39%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (1H, s), 7.75 (0.5H, s), 7.71 (0.5H, s), 7.53–7.39 (6H, m), 7.31–7.20 (8H, m), 6.81–6.78 (4H, m), 6.51 (1H, dd, *J*=7.9, 5.2 Hz), 4.68 (0.5H, d, *J*=4.0 Hz), 4.59 (0.5H, d, *J*=3.1 Hz), 4.36–4.27 (1.5H, m), 4.13–4.08 (0.5H, m), 3.78–3.77 (6H, m), 3.75–3.65 (1H, m), 3.62–3.35 (4H, m), 3.27–3.22 (1H, m), 2.88–2.81 (1H, m), 2.69–2.62 (1H, m), 2.56–2.53 (1H, m), 2.36–2.32 (1H, m), 2.00 (1.5H, s), 1.98 (1.5H, s), 1.12 (3H, d, *J*=6.7 Hz), 1.09–1.06 (6H, m), 0.92 (3H, d, *J*=6.7 Hz). ^{31}P NMR (161 MHz, $CDCl_3$): δ 150.3, 149.7. ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.3, 158.5, 150.3, 144.6, 144.5, 139.0, 135.8, 134.8, 134.7, 130.2, 128.3, 127.8, 126.8, 126.3, 126.2, 120.7, 117.6, 117.4, 113.1, 111.5, 111.4, 92.1, 92.0, 87.8, 87.3, 86.2, 85.8, 85.7, 81.1, 73.7, 73.6, 72.7, 72.6, 63.3, 62.5, 58.7, 58.6, 58.2, 58.1, 55.3, 55.2, 48.6, 48.4, 43.5, 43.4, 43.3, 31.6, 24.6, 24.5, 24.4, 22.6, 22.4, 20.3, 20.2, 14.1, 12.8. FTIR (neat): 2967, 1691 cm^{-1}. ESI-MS *m*/*z*: 996 $(M+Na)^+$.

*4.1.15. (1′S,3′R,4′R,5′R,7′S)-N^4-Benzoyl-1-{1′-(p-trifluoroacetylaminophenyl)-3′-dimethoxytrityloxymethyl-4′-O-(N,N-diisopropyl-β-cyanoethyl-phosphoramidyl)-2′,6′-dioxabicyclo[3.3.0]oct-7′-yl}-cytosine (**9b**-**3**)*

A colorless foam (59%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.63 (1H, s), 8.13 (0.5H, d, *J*=7.3 Hz), 7.94–7.90 (3H, m), 7.68 (1H, d, *J*=8.6 Hz), 7.63–7.59 (2H, m), 7.52–7.41 (6H, m), 7.32–7.17 (8H, m), 6.81–6.78

(4H, m), 6.36 (0.5H, t, J=7.0 Hz), 6.23 (0.5H, t, J=6.7 Hz), 5.06 (0.5H, d, J=3.4 Hz), 4.98 (0.5H, d, J=3.4 Hz), 4.41–4.35 (0.5H, m), 4.31–4.25 (1H, m), 4.20–4.15 (0.5H, m), 3.78 (3H, s), 3.77 (3H, s), 3.76–3.67 (1H, m), 3.60–3.36 (4H, m), 3.29–3.18 (2H, m), 2.65–2.55 (1.5H, m), 2.49 (0.5H, dd, J=14.3, 7.6 Hz), 2.39–2.29 (1H, m), 1.12 (3H, d, J=6.7 Hz), 1.07 (4H, d, J=6.7 Hz), 0.92–0.85 (5H, m). ^{31}P NMR (161 MHz, $CDCl_3$): δ 150.3, 149.6. ^{13}C NMR (125 MHz, $CDCl_3$): δ 158.5, 144.7, 144.6, 138.4, 138.2, 138.0, 136.0, 135.9, 134.6, 133.2, 130.3, 129.1, 128.4, 127.7, 127.5, 126.8, 126.7, 126.6, 120.6, 117.9, 117.4, 113.1, 92.1, 91.9, 90.7, 88.8, 88.6, 86.1, 81.1, 81.0, 62.9, 62.0, 58.8, 58.6, 58.0, 57.9, 55.2, 49.9, 49.1, 43.4, 43.3, 43.2, 24.6, 24.5, 20.4, 20.1. FTIR (neat): 2964, 2930, 1706, 1660, 1609 cm^{-1}. ESI-MS m/z: 974 $(M+H)^+$, 1086 $(M+Na)^+$.

*4.1.16. (1′S,3′R,4′R,5′R,7′R)-N^4-Benzoyl-1-{1′-(p-trifluoroacetyl-aminophenyl)-3′-dimethoxytrityloxymethyl-4′-O-(N,N-diisopropyl-β-cyanoethyl-phosphoramidyl)-2′,6′-dioxabicyclo[3.3.0]oct-7′-yl}-cytosine (**9a**-**3**)*

A colorless foam (35%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.68 (1H, s), 8.49–8.47 (1H, m), 8.09 (1H, s), 7.88 (2H, d, J=7.3 Hz), 7.62–7.58 (1.5H, m), 7.52–7.46 (6H, m), 7.37–7.35 (2H, m), 7.26–6.17 (7.5H, m), 6.81–6.75 (4H, m), 6.38–6.31 (1H, m), 4.92–4.89 (0.5H, m), 4.81 (0.5H, d, J=4.6 Hz), 4.53–4.47 (0.5H, m), 4.19–4.14 (1.5H, m), 3.77–3.76 (6H, m), 3.60–3.33 (5H, m), 3.27–3.02 (2H, m), 2.85–2.71 (1.5H, m), 2.60–2.56 (0.5H, m), 2.36–2.32 (1H, m), 1.17–1.12 (6H, m), 1.05–1.04 (2H, m), 0.88–0.84 (4H, m). ^{31}P NMR (161 MHz, $CDCl_3$): δ 150.0, 149.9. ^{13}C NMR (125 MHz, $CDCl_3$): δ 158.5, 144.6, 135.8, 133.3, 133.2, 130.2, 129.1, 128.3, 128.2, 127.8, 127.5, 126.9, 126.8, 126.3, 126.2, 120.8, 120.7, 113.2, 113.1, 88.6, 62.2, 55.3, 55.2, 49.9, 46.5, 45.3, 43.5, 43.4, 24.7, 24.6, 23.0, 22.9, 22.6, 22. FTIR (neat): 2967, 2930, 1703, 1660, 1609 cm^{-1}. ESI-MS m/z: 1064 $(M+H)^+$.

4.2. Synthesis of TFOs containing *p*-amino-WNA derivatives

Triplex forming oligonucleotides (TFOs) incorporating the *p*-amino-WNA analogs were synthesized using an automated DNA synthesizer (Applied Biosystems 394 DNA/RNA Synthesizer). After cleavage from CPG, they were purified by HPLC (HPLC conditions: COSMOSIL 5C18-MS-II (10×250 mm), buffer A: 0.1 M TEAA, buffer B: CH_3CN, 10–40%/20 min, 40–100%/ 30 min linear gradient, flow rate: 4.0 mL/ min, UV-detector: 254 nm, column oven: 35 °C). All synthesized TFOs were identified by MALDI-TOF MS measurement. *MALDI-TOF MASS results: TFOs having p-amino-WNA-βT.* **TFO1**(**βT**): calcd for 5873.05, found 5873.68; **TFO2**(**βT**): calcd for 5889.04, found 5889.83; **TFO3**(**βT**): calcd for 5857.06, found 5856.29; **TFO4**(**βT**): calcd for 5873.05, found 5873.88. *TFOs having p-amino-WNA-αT.* **TFO1**(**αT**): calcd for 5873.05, found 5869.52; **TFO2**(**αT**): calcd for 5889.04, found 5889.53; **TFO3**(**αT**): calcd for 5857.06, found 5856.19; **TFO4**(**αT**): calcd for 5873.05, found 5872.54. *TFOs having p-amino-WNA-βC.* **TFO1**(**βC**): calcd for 5858.05, found 5857.70; **TFO2**(**βC**): calcd for 5874.04, found 5873.78; **TFO3**(**βC**): calcd for 5842.06, found 5841.18; **TFO4**(**βC**): calcd for 5858.05, found 5857.75. *TFOs having p-amino-WNA-αC.* **TFO1**(**αC**): calcd for 5858.05, found 5856.82; **TFO2**(**αC**): calcd for 5874.04, found 5873.32; **TFO3**(**αC**): calcd for 5842.06, found 5841.70; **TFO4**(**αC**): calcd for 5858.05, found 5857.67.

4.3. Gel-shift assay

Triplex formation was done for 12 h at 22 °C in the buffer containing 20 mM Tris–HCl, 5 mM $MgCl_2$, 2.5 mM spermidine, and 10% sucrose at pH 7.5. Electrophoresis was done at 10 °C with a non-denatured polyacrylamide gel. TFO (10 nM) containing the ^{32}*P*-labeled one as the tracers was used.[6,9] The association constant was calculated using the concentration of each component and the following equation: K_s=[triplex]/[duplex][TFO].

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References and notes

1. (a) Ye, Z.; Guntaka, R. V.; Mahato, R. I. *Biochemistry* **2007**, *46*, 11240–11252; (b) Alam, M. R.; Majumbar, A.; Thazhathveetil, A. K.; Liu, S.-T.; Liu, J.-L.; Puri, N.; Cuenoud, B.; Sasaki, S.; Miller, P. S.; Seidman, M. M. *Biochemistry* **2007**, *46*, 10222–10233; (c) Hewett, P. W.; Daft, E. L.; Laughton, C. A.; Ahmad, S.; Ahmad, A.; Murray, C. *Mol. Med.* **2006**, *12*, 8–16; (d) Rogers, F. A.; Lloyd, J. A.; Glazer, P. M. *Curr. Med. Chem. Anti-Cancer Agents* **2005**, *5*, 319–326; (e) Besch, R.; Giovannangeli, C.; Degitz, K. *Curr. Drug Targets* **2004**, *5*, 691–703.
2. (a) Ghosh, M. K.; Katyal, A.; Chandra, R.; Brahmachari, V. *Mol. Cell. Biochem.* **2005**, *278*, 147–155; (b) Song, J.; Intody, Z.; Li, M.; Wilson, J. H. *Gene* **2004**, *324*, 183–190; (c) Stanojevic, D.; Young, R. A. *Biochemistry* **2002**, *41*, 7209–7216.
3. Kalish, J. M.; Seidman, M. M.; Weeks, D. L.; Glazer, P. M. *Nucleic Acids Res.* **2005**, *33*, 3492–3502.
4. Soyfer, V. N.; Potaman, V. N. *Triple-Helical Nucleic Acids*; Springer: New York, NY, 1996; and references cited therein.
5. Sasaki, S.; Yamauchi, H.; Nagatsugi, F.; Takahashi, R.; Taniguchi, Y.; Maeda, M. *Tetrahedron Lett.* **2001**, *42*, 6915–6918.
6. Sasaki, S.; Taniguchi, Y.; Takahashi, R.; Senko, Y.; Kodama, K.; Nagatsugi, F.; Maeda, M. *J. Am. Chem. Soc.* **2004**, *126*, 516–528.
7. Taniguchi, Y.; Nakamura, A.; Senko, Y.; Kodama, K.; Nagatsugi, F.; Sasaki, S. *Nucleosides Nucleotides Nucleic Acids* **2005**, *24*, 823–827.
8. Taniguchi, Y.; Nakamura, A.; Aoki, E.; Sasaki, S. *Nucleic Acids Symp. Ser.* **2005**, *49*, 173–174.
9. Taniguchi, Y.; Nakamura, A.; Senko, Y.; Nagatsugi, F.; Sasaki, S. *J. Org. Chem.* **2006**, *71*, 2115–2122.
10. The example of interaction between an amino group and a phosphate group: Buchini, S.; Leumann, C. J. *Tetrahedron Lett.* **2003**, *44*, 5065–5068.
11. Albrecht, M.; Song, Y. *Synthesis* **2006**, *18*, 3037–3042.
12. Bindal, R. D.; Katzenellenbogen, J. A. *J. Org. Chem.* **1987**, *52*, 3181–3185.
13. Sadeghi-Khomami, A.; Blake, A. J.; Thomas, N. R. *Org. Lett.* **2005**, *7*, 4891–4894.
14. Ninomiya, K.; Shioiri, T.; Yamada, S. *Tetrahedron* **1974**, *30*, 2151–2157.
15. (a) Schreivogel, A.; Maurer, J.; Winter, R.; Baro, A.; Laschat, S. *J. Org. Chem.* **2006**, *71*, 3395–3404; (b) Matulic-Adamic, J.; Beigelman, L. *Tetrahedron Lett.* **1996**, *37*, 6973–6976.
16. (a) Griffin, L. C.; Kiessling, L. L.; Beal, P. A.; Gillepie, P. B.; Dervan, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 7976–7982; (b) Wang, E.; Koshlap, K. M.; Gillespie, P.; Dervan, P. B.; Feigon, J. *J. Mol. Biol.* **1996**, *257*, 1052–1069.